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RESEARCH ARTICLE

# Conceptual basis for studying the fungal biodiversity in organogenic soils in the area of the Bulgarian Antarctic Base 'St. Kliment Ohridski' on Livingston Island

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## Abstract

In 2023, a scientific project for studying the fungal biodiversity in organogenic soils in the area of the Bulgarian Antarctic Base 'St. Kliment Ohridski' on Livingston Island was funded by the National Center for the Polar Studies at Sofia University 'St. Kliment Ohridski', implemented under the National Program for Polar Research 'From Pole to Pole', adopted by the Ministry of Education and Science.

In the period February-March 2023, a total of 126 soil samples were collected for analysis. Soil samples were carried out in the upper 0-5 cm soil layer. In places with deeper soil profiles, samples were collected from the deeper layer >5 cm. All samples were stored in sterile polyethylene bags in a freezer at minus 28°C on the Bulgarian ship 'St. St. Cyril and Methodius', which arrived in Bulgaria on 2nd May 2023.

The present study aims to present the conceptual basis for the study of structure, composition, and bioecological features of fungal species occurring in Antarctic organogenic soils from Livingston Island, to investigate the representatives of separate taxonomic groups of invertebrate organisms interacting with the fungal species in the soil, and to establish the relationships with the soil characteristics in the studied sites. Studies of the biological and ecological features of representatives of the mycota in Antarctic organogenic soils, their physiological and biochemical behavior, and interaction with invertebrate organisms, are essential for a better understanding of the biological processes occurring in them, and the implementation of this project will have substantial contribution to these issues.

## Keywords

Organogenic soils, fungal species, soil characteristics, Livingston Island, Antarctica

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Antarctica is an unexplored environment that presents a unique opportunity to conduct taxonomic, ecological and biotechnological research on microorganisms. For decades, the existence of insects, mites, and other invertebrates on the Antarctic continent has been of great interest to many researchers (Gressitt, 1967; Brunetti et al., 2021). They are important in simple food chains that include segments of available flora and microorganisms and play a role in soil formation.

The study of living organisms in Antarctic soils represents one of the most highly prioritized areas of research (Ivanova et al., 2021). The essential role is occupied by studies on microorganisms and their role in the course of biotic processes and the functioning of the soil system. At the same time, in recent years research on microorganisms adapted to extreme climatic conditions has increased, driven by the possibility of their application in biotechnology. Technological advances in molecular biology research indicate the presence of endemic species in Antarctica, which are a potential source of new, important biotechnological compounds for their application in medicine for example, in the creation of new antibiotics (Hughes et al., 2015; Abneuf et al., 2016; Wang et al., 2013; Núñez-Montero, Barrientos, 2018; Silva et al., 2020; Varrella et al., 2021).

In recent years, microbiological analyses have been carried out in polar soils at selected monitoring sites on Livingston Island, where the quantitative and qualitative composition of the heterotrophic block of soil microflora (non-sporing bacteria, gram-positive bacteria, actinomycetes, micromycetes, mineral nitrogen-absorbing bacteria) has been determined. Aerobic and anaerobic groups of soil microorganisms were studied, and the biogenicity and rate of mineralization processes in organogenic soils were determined (Malcheva et al., 2020).

Organic soils are characteristic of the ecosystems of the Southern Hemisphere, representing the most extensive source of organic matter for the terrestrial ecosystem of Antarctica (Abakumov, 2010). In the last decade, several studies have focused on the ornithogenic factor of soil formation under Antarctic conditions. In recent years, microbiological analyses have been carried out in organogenic soils in permanent sample plots for monitoring the coastal zone in the area of the Bulgarian Antarctic Base (BAB) 'St. Kliment Ohridski' on Livingston Island (Malcheva et al., 2020, Abakumov et al., 2022; Malcheva et al., 2022, etc.), conservation of biodiversity to mitigate the negative impact of human activity and a changing climate. The obtained results contribute to the study of the microbiota of the organic soils of Antarctica and the adaptive abilities of the microbiota to the harsh conditions of the ecosystems of the polar regions.

The presence of fungal mycota in Antarctic organic soils is of particular interest to researchers due to the great diversity of this microgroup. For this reason, research on cold-adapted microorganisms has increased in recent years, driven by their potential for applications in biotechnology. Prior to the advent of the polymerase chain reaction (PCR) and more readily available sequencing technologies in the 1980s and 1990s, taxonomic identification of fungal isolates was primarily based on morphological characteristics (Arenz et al., 2014).

The predominant species are from the division Ascomycota and their anamorphic stages, followed by the representatives of Basidiomycota and Zygomycota (Rosa et al., 2019). The established fungal diversity on King George, Deception, and Robert islands includes 54 species of fungi – representatives of separate taxonomic groups (Durán et al., 2019), 309 species on Collins Glacier on King George Island (Santos et al., 2020), etc.

The adaptation of fungi to climate change and the assessment of their diversity and distribution in organic soils represent an interesting object for monitoring the effects of global climate change on these territories. Studies of the biological and ecological features of representatives of the mycota in Antarctic organogenic soils, their physiological and biochemical behavior, their interaction with invertebrate organisms, are essential for a better understanding of the biological processes occurring in them, and the implementation of this project will have substantial contribution to this.

Analyzing the conducted research and the results in the scientific database, it should be noted that there is a lack of targeted and in-depth studies on the identification of the fungal diversity distributed in the organogenic soils in the BAB 'St. Kliment Ohridski' area of Livingston Island.

In 2023, a scientific project for studying the fungal biodiversity in organogenic soils in the area of the Bulgarian Antarctic Base 'St. Kliment Ohridski' on Livingston Island was financed by the National Center for the Polar Studies at Sofia University 'St. Kliment Ohridski', implemented under the National Program for Polar Research 'From Pole to Pole', adopted by the Ministry of Education and Science. Funding under the current procedure is aimed to support the implementation of non-profit scientific activity for scientific research to acquire new knowledge about the polar regions of the Earth.

The aim of the present study was focused on the general concept to identify and analyse the structure, composition, cultural (morphological and phenotypic), and physiological features of fungal species diversity occurring in Antarctic organogenic soils from Livingston Island, to study representatives of separate taxonomic groups of invertebrate organisms interacting with the fungal species in the soil, and to establish the main soil characteristics in the studied monitoring sites.

In the implementation of the current activities, tasks related to the upgrading of the results obtained from previous expeditions on the Livingstone Island (2018 and 2019); clarification of the complex of fungal diversity forming the mycota in the studied sites; determining the role of invertebrate species for the spread of fungi in the soil; collecting information on soil indicators and supplementing available databases; determination of basic soil characteristics; visualization of the distribution of fungal biodiversity through mapping in GIS. The project includes interdisciplinary studies, which justified the attraction of experts with competence in separate scientific fields.

In the period February-March 2023, a total number of 126 soil samples were collected for analysis. Soil samples were carried out in the upper 0-5 cm soil layer (Figure 1, Table 1). In places with deeper soil profiles, samples were collected from the deeper layer >5 cm. All collected samples were stored in sterile polyethylene bags in a freezer at minus 28°C on the Bulgarian ship 'St. St. Cyril and Methodius', which arrived in Bulgaria on 2nd May 2023.

**Figure 1.** Livingston Island (Ivanov, 2017, left) and studied plots for soil sampling in 2023 (right)

**Table 1.** Characteristics of studied plots on Livingston Island in 2023

| Sample plots | Date | Location | Coordinates | | Altitude [m] | Depth [cm] | Number of samples |
|---|---|---|---|---|---|---|---|
| | | | S | W | | | |
| Liv 1 new | 01.03.2023 | Caletta Agrentina | 62°40'3.13" | 60°24'4.89" | -9 | 0-5 | 10 |
| Liv 2 old (2018; 2019) | 01.03.2023 | Caletta Agrentina | 62°40'1.28" | 60°24'1.25" | -7 | 0-5 | 9 |
| Liv 3 new | 03.03.2023 | Sally Rocks | 62°42'13.3" | 60°25'13.5" | 4 | 0-5 | 8 |
| Liv 4 old (2019) | 19.02.2023 | Green Peace | 62°38'2.45" | 60°21'6.18" | 9 | 0-5 | 10 |
| Liv 5 old (2018; 2019) | 19.02.2023 | Green Peace | 62°38'11.0" | 60°21'2.42" | 0 | 0-5 | 10 |
| Liv 6 new | 22.02.2023 | Spain base | 62°39'7.36" | 60°22'7.05" | 32 | 0-10 | 13 |
| Liv 7 new | 22.02.2023 | Spain base | 62°39'7.36" | 60°23'55.6" | -7 | 0-5 | 8 |
| Liv 8 new | 26.02.2023 | Johnsons Dock | 62°39'4.94" | 60°21'8.32" | 0 | 0-10 | 11 |
| Liv 9 new | 26.02.2023 | Johnsons Dock | 62°39'6.57" | 60°21'34.3" | 0 | 0-5 | 8 |
| Liv 10 new | 24.02.2023 | Isperides Peak | 62°38'6.73" | 60°22'2.19" | 89 | 0-10 | 5 |
| Liv 11 new | 24.02.2023 | over the Mongolian Gulf | 62°38'8.32" | 60°22'3.02" | 35 | 0-10 | 9 |
| Liv 12 new | 26.02.2023 | Sinemorets Peak | 62°38'38.9" | 60°21'5.91" | 56 | 0-10 | 5 |
| Liv 13 new | 26.02.2023 | Sinemorets Peak | 62°38'33.6" | 60°21'5.85" | 26 | 0-10 | 4 |
| Liv 14 new | 04.03.2023 | Perunika Glacier | 62°38'0.76" | 60°20'9.83" | 3 | 0-5 | 8 |
| Liv 15 new | 03.03.2023 | Hannah Point | 62°38'8.85" | 60°35'2.40" | -11 | 0-5 | 8 |
| Total samples: | | | | | | | 126 |

Although molecular biology methods represent the recently applied taxonomic tool for the identification of fungi from Antarctic soils, as a first step in the present study classical macro- and micromorphological and physiological methods will be

applied to determine the taxonomic structure of the mycota representatives found in the samples taken.

To determine the composition and structure of the fungal diversity in the studied soil samples, an average sample weighing 3 g and dissolved in 10 ml of distilled water will be used. Using the Sartorius Membrane Filter, 2 ml of the resulting solution will be filtered. Three non-perishable microscope slides will be prepared from the precipitate retained on the filter paper. Each preparation will be microscopically examined with a Zeiss NU2 light microscope, determining morphological characteristics and the number of spores occurring per 1 $cm^2$ area at ×125, 250, and 312.5 times magnification.

The identification of the fungal organisms from each soil sample will be carried out according to the methodology described by Gonc et al. (2015): 1 g of soil from each sample was added to 9 ml of disinfectant solution (0.85% NaCl) in triplicate and vortexed. One hundred microliters of the homogenized soil will be placed on an artificial culture medium (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 2% glucose, 2% agar, pH 6.2±2, antibiotic solution). The inocula will be incubated in a thermostat at different temperature regimes: -4, 0, 4, and 10°C for 30 days to evaluate the optimal growth temperature of the mycelial isolates of the fungal organisms. Different nutrient media (potato dextrose agar, malt agar, Sabouraud's dextrose, and yeast extract sucrose agars) will be tested to determine the physiological and cultural characteristics of the fungal species.

For the species identification of the fungi in the studied samples, DNA metagenomic sequencing will be performed in specialized laboratories equipped with the necessary equipment.

In the entomology laboratory, soil samples will be processed to determine the composition and structure of invertebrate organisms and their interrelationship with established fungal organisms. The laboratory is equipped with a Leica MS5 stereomicroscope, complete with a Leica DFC295 digital camera; heated insectarium, portable digital cameras; electronic scale Metler AE 200; freezer, refrigerators, etc.

The chemical and physical properties of the soil samples will be investigated in the soil science laboratories equipped with specialized laboratory equipment: ICP OS, Perkin Elmer Atomic Absorption Spectrophotometer, water distiller, electronic instruments, C and N determination equipment, UV-VIS spectrophotometer.

The activities planned for the project include the implementation of interdisciplinary research related to the identification of fungal diversity and species of invertebrate organisms inhabiting Livingstone island organic soils, research in the field of soil science, ecology, mapping in a GIS environment, etc. To achieve the set goal and to ensure interdisciplinary research, the team of scientists includes experts from different scientific fields. With its scientific competence, the team will ensure high-quality science-based implementation of the project by consistently following the activities set out in the work program, presenting the obtained results at international conferences, and publishing them in prestigious journals with an impact factor or impact rank, creating accessible databases, etc.

For the first time in Bulgaria, an interdisciplinary approach will be applied to identify the fungal diversity in organogenic soils from representative sites on Livingston Island, based on conducting classic macro- and micro-research, as well as applying innovative methods and technologies for molecular research. Studies of the biological and ecological features of representatives of the mycota in Antarctic organic soils, their physiological and biochemical behaviour, their interaction with invertebrate organisms, are essential for a better understanding of the biological processes occurring in them.

The conceptual basis ensures provisioning of results that will contribute to expanding the knowledge of the fungal species occurring in Antarctic organogenic soils on Livingston Island. Our understanding of the complex biochemical processes that determine the existence of these species under certain soil characteristics and climatic conditions will benefit just as well as our ability to clarify the interrelationships of individual representatives of the mycota with the occurring invertebrate species in the studied habitats, which are representative from the point of view of the lack of the human factor and the influence of climate changes for the polar regions as well.

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